MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2020

*April–June 2020—Volume 135, pp. 371–382*

https://doi.org/10.5248/135.371

# *Vanderbylia cinnamomea* sp. nov. from southwestern China

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Abstract—*Vanderbylia cinnamomea* is proposed as a new wood-inhabiting polypore species based on morphological characters supported by phylogenetic analysis. The species is characterized by an annual, pileate basidiocarp, a brown to black pileal surface, an olivaceous buff to smoke grey pore surface, a dimitic hyphal system with clamped generative hyphae, and subglobose to amygdaliform, hyaline, thick-walled, smooth, dextrinoid, and cyanophilous basidiospores. ITS+nLSU sequence analyses place *V. cinnamomea* in *Vanderbylia* within a subclade with a high support (100% BS, 100% BP, 1.00 BPP) sister to a subclade comprising *V. fraxinea* and *V. robiniophila*.

Key words—*Perenniporia*, *Polyporaceae*, *Polyporales*, taxonomy, Yunnan Province

## Introduction

*Vanderbylia* D.A. Reid (*Polyporaceae*, *Polyporales*) is typified by *V. vicina* (Lloyd) D.A. Reid (Reid 1973). Ryvarden & Johansen (1980) reduced the genus to a synonym under *Perenniporia* Murrill for a long time, but recent phylogenetic research of a combined sequence dataset of the internal transcribed spacer (ITS) and the large subunit nuclear ribosomal RNA gene (nLSU) supports *Vanderbylia* as an independent genus (Robledo &

al. 2009, Zhao & al. 2013, Cui & al. 2019). Morphologically *Vanderbylia* is characterized by pileate basidiocarps, a dimitic hyphal system with clamped generative hyphae and variably dextrinoid skeletal hyphae, and subglobose to amygdaliform, non-truncate and variably dextrinoid basidiospores (Reid 1973, Zhao & Cui 2013). Currently, nine species are accepted in *Vanderbylia* worldwide: *V. borneensis* Corner, *V. delavayi* (Pat.) B.K. Cui & Y.C. Dai, *V. devians* (Bres.) D.A. Reid, *V. fraxinea* (Bull.) D.A. Reid, *V. nigroapplanata* (Van der Byl) D.A. Reid, *V. robiniophila* (Murrill) B.K. Cui & Y.C. Dai, *V. subincarnata* Corner, *V. ungulata* D.A. Reid, and *V. vicina* (Bresadola 1920, Reid 1975, Corner 1987, Gilbertson & Ryvarden 1987, Núñez & Ryvarden 2001, Ryvarden & Melo 2014, Cui & al. 2019).

Robledo & al. (2009), who revealed the relationship between *Perenniporia* and *Perenniporiella* Decock & Ryvarden, grouped two *Vanderbylia* species together—the generic type *V. vicina* and *V. fraxinea*. In their phylogenetic overview of *Perenniporia* s.l., Zhao & al. (2013) and revealed that *Vanderbylia fraxinea*, *V. robiniophila,* and *V. vicina* form a single lineage. Subsequently, Cui & al. (2019) investigated the species diversity, taxonomy, and phylogeny of *Polyporaceae* in China and proposed two new combinations, *Vanderbylia delavayi* and *V. robiniophila*.

During investigations on wood-inhabiting fungi in Yunnan Province, an additional taxon was found that could not be assigned to any described species. In examining the taxonomy and phylogeny of this new species, we employed a two-gene molecular phylogenetic approach using internal transcribed spacer (ITS) and long subunit (nLSU) plus an expanded sampling of *Vanderbylia* isolates that support the existence of a new species, *Vanderbylia cinnamomea.*

**Materials & methods**

The specimens studied are deposited at the herbarium of Southwest Forestry University, Kunming, P.R. China (SWFC). Macromorphological descriptions are based on field notes. Color terms follow Petersen (1996). Micromorphological data were obtained from the dried specimens and observed under light microscopy following Dai (2012). The following abbreviations are used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = both non-amyloid and non-dextrinoid, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, n (a/b) = number of spores (a) measured from given number (b) of specimens.

We extracted genomic DNA from dried specimens using Magen Biotech HiPure Fungal DNA Mini Kit II according to the manufacturer's instructions with some

Table 1. Species, specimens, and sequences used in this study.
(New sequences in **bold**).

| Species name | Sample no. | GenBank no. | | References |
|---|---|---|---|---|
| | | ITS | nLSU | |
| *Donkioporia expansa* | MUCL 35116 | FJ411104 | FJ393872 | Robledo & al. (2009) |
| *Pyrofomes demidoffii* | MUCL 41034 | FJ411105 | FJ393873 | Robledo & al. (2009) |
| ***Vanderbylia cinnamomea*** | **CLZhao 8952 T** | MT372778 | MT372788 | Present study |
| | **CLZhao 8959** | MT372779 | MT372789 | Present study |
| | **CLZhao 8961** | MT372780 | MT372790 | Present study |
| | **CLZhao 8962** | MT372781 | MT372791 | Present study |
| | **CLZhao 8963** | MT372782 | MT372792 | Present study |
| | **CLZhao 9001** | MT372783 | MT372793 | Present study |
| *V. fraxinea* | DP 83 | AM269789 | AM269853 | Robledo & al. (2009) |
| | Cui 7154 | HQ654095 | HQ654110 | Zhao & al. (2013) |
| | Cui 8871 | JF706329 | JF706345 | Zhao & al. (2013) |
| *V. robiniophila* | Cui 5644 | HQ876609 | JF706342 | Zhao & al. (2013) |
| | Cui 7144 | HQ876608 | JF706341 | Zhao & al. (2013) |
| | Cui 9174 | HQ876610 | JF706343 | Zhao & al. (2013) |
| *V. vicina* | MUCL 44779 | FJ411095 | AF518666 | Robledo & al. (2009) |

modifications. A small piece (about 30 mg) of dried fungal material was ground to powder with liquid nitrogen, transferred to a 1.5 mL centrifuge tube, suspended in 0.4 mL of lysis buffer, and incubated in a 65 °C water bath for 60 min. After the addition of 0.4 mL phenol-chloroform (24:1) to each tube, the suspension was shaken vigorously. After centrifugation at 13,000 rpm for 5 min, 0.3 mL supernatant was transferred to a new tube and mixed with 0.45 mL binding buffer. That mixture was transferred to an adsorbing column (AC) for centrifugation at 13,000 rpm for 0.5 min. Then, 0.5 mL inhibitor removal fluid was added in AC for a centrifugation at 12,000 rpm for 30 s. After washing twice with 0.5 mL washing buffer, the AC was transferred to a clean centrifuge tube, and 0.1 mL elution buffer was added to the middle of adsorbed film to elute the genomic DNA. The ITS region was amplified with primer pairs ITS5 and ITS4 (White & al. 1990). Nuclear LSU region was amplified with primer pairs LR0R and LR7 (https://sites.duke.edu/vilgalyslab/rdna_primers_for_fungi). The PCR procedure for ITS was optimized as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles at 94 °C for 40 s, 58 °C for 45 s, and 72 °C for 1 min, and a final extension of 72 °C for 10 min. The PCR condition for nLSU was as follow: initial denaturation at 94 °C for 1 min, followed by 35 cycles at 94 °C for 30 s, 48 °C 1 min and 72 °C for 1.5 min, and a final extension of 72 °C for 10 min. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company. The six new sequences from specimens of *Vanderbylia cinnamomea* were aligned with additional *Vanderbylia* sequences downloaded from GenBank (Table 1).

Sequences were aligned in MAFFT 7 (http://mafft.cbrc.jp/alignment/server/) using the "G-INS-I" strategy, and manually adjusted in BioEdit (Hall 1999).

Alignment datasets were deposited in TreeBase (submission ID 24081). GenBank sequences of *Donkioporia expansa* (Desm.) Kotl. & Pouzar and *Pyrofomes demidoffii* (Lév.) Kotl. & Pouzar obtained from GenBank were used as an outgroup to root trees following Zhao & al. (2013) in the ITS+nLSU analysis.

Maximum parsimony analysis was applied to the ITS+nLSU dataset sequences. The tree construction procedure was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted, and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BP) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree generated. Maximum Likelihood (ML) analysis with RAxML-HPC2 was conducted for ITS datasets on Abe through the Cipres Science Gateway (www.phylo.org; Miller & al. 2009). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicate.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI). Bayesian inference was calculated with MrBayes_3.1.2 with a general time reversible (GTR+I+G) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 2 million generations and trees were sampled every 100 generations. The first one-fourth generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for greater than or equal to 80% (maximum likelihood, BL), 50% (maximum parsimony, BP), and 0.95 (Bayesian posterior probabilities, BPP) were considered as significantly supported.

**Phylogenetic results**

The ITS dataset included sequences from 15 fungal specimens or isolates representing 6 species. The dataset had an aligned length of 1844 characters, of which 1690 characters were constant, 65 were variable and parsimony-uninformative, and 89 were parsimony-informative. Maximum parsimony analysis yielded 6 equally parsimonious trees (TL = 178, CI = 0.921, HI = 0.787, RI = 0.944, RC = 0.870). Best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis was GTR+I+G. Bayesian and ML analyses produced a similar topology as MP analysis, with an average standard deviation of split frequencies = 0.001512 (BI).

The phylogeny (Fig. 1) inferred from ITS+nLSU sequences obtained for related taxa of *Vanderbylia* support the new species as independent in one sub-clade and sister to the /fraxinea-robinophila subclade.

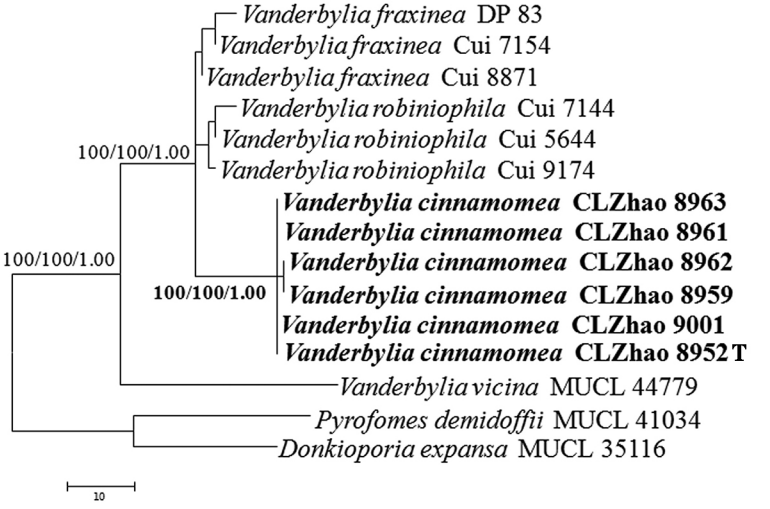


Fig. 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Vanderbylia cinnamomea* and related species in *Vanderbylia* based on ITS+nLSU sequence analyses. Branches are labeled with maximum likelihood bootstrap >70%, parsimony bootstrap proportions >50% and Bayesian posterior probabilities >0.95.

## Taxonomy

***Vanderbylia cinnamomea*** C.L. Zhao, **sp. nov.** Figs 2–4

MB 831788

Differs from *Vanderbylia vicina* by its annual basidiocarps and its smaller basidiospores.

Type: China. Yunnan Province: Kunming, Tanhuasi Park, on living tree of *Cinnamomum camphora* (L.) J. Presl (*Lauraceae*), 13 October 2018, CL Zhao 8952 (**Holotype**, SWFC 008952; GenBank MT372778, MT372788).

Etymology: The specific epithet *cinnamomea* (Lat.) refers to the host of *Cinnamomum camphora*.

Basidiomata annual, pileate, imbricate, without odor or taste and corky when fresh, becoming hard corky upon drying. Pilei dimidiate, projecting ≤10 cm, 18 cm wide, 4.5 cm thick at centre. Pileal surface pale brown to brown when fresh and brown to black from the base upon drying, smooth, gently concentrically sulcate, glabrous. Pore surface pure buff to olivaceous buff when fresh, olivaceous buff to smoke grey upon drying; pores round, 5–7 per mm; dissepiments thick, entire. Sterile margin narrow, buff, ≤0.5

mm wide. Context cream, cottony, ≤4 cm thick. Tubes concolorous with pore surface, ≤5 mm long.

Hyphal structure dimitic; generative hyphae with clamp connections, strongly dextrinoid, CB+; tissues unchanging in KOH.

Context generative hyphae infrequent, hyaline, thin-walled, unbranched, 2–3 μm in diam; skeletal hyphae dominant, hyaline, thick-walled with a wide to narrow lumen, branched, interwoven, 4.5–5.5 μm in diam.

Tubes generative hyphae infrequent, hyaline, thin-walled, unbranched, 1.5–2.5 μm in diam; skeletal hyphae dominant, hyaline, thick-walled with a wide to narrow lumen, branched, interwoven, 4–5 μm in diam. Presence of arboriform hyphae, thick-walled to almost solid, with few branches and short, lateral or terminal processes, tortuous, 25–40 × 2–6 μm. Cystidia and cystidioles not seen; basidia clavate, with 4 sterigmata and a basal clamp connection, 20–24 × 6–9.5 μm; basidioles dominant, clavate-shaped to pear-shaped.

Basidiospores subglobose to amygdaliform, hyaline, thick-walled, smooth, strongly dextrinoid, cyanophilous, 5–6(–6.5) × 4–5(–5.5) μm, L = 5.67 μm, W = 4.73 μm, Q = 1.17–1.21 (n = 180/6).

Type of rot: white.

Additional specimens examined: **CHINA. Yunnan province. Kunming:** Tanhuasi Park, on living tree of *Cinnamomum camphora,* 15 October 2018, CLZhao 8959 (SWFC 008959; GenBank MT372779, MT372789); CLZhao 8961 (SWFC 008961; GenBank MT372780, MT372790); CLZhao 8962 (SWFC 008962, GenBank MT372781, MT372791); CLZhao 8963 (SWFC 008963; GenBank MT372782, MT372792); CLZhao 9001 (SWFC 009001; GenBank MT372783, MT372793).

## Discussion

We describe here a new species *Vanderbylia cinnamomea* based on phylogenetic analyses and morphological characters.

Phylogenetically, *Vanderbylia cinnamomea* grouped with species *V. fraxinea, V. robiniophila,* and *V. vicina* in the rDNA-based phylogeny (Fig. 1). Morphologically *V. fraxinea* differs in its perennial basidiocarps and larger basidiospores (6–8 × 5–6.5 μm, Ryvarden & Melo 2014), while *Vanderbylia robiniophila* is distinguished by its growth on *Robinia* L., its white to pale brown pore surface, and its larger basidiospores (5–8 × 5–7 μm, Gilbertson & Ryvarden 1987; Núñez & Ryvarden 2001); *Vanderbylia vicina* differs in its perennial basidiocarps and larger basidiospores (8–8.9 × 6.9–7.5 μm, Reid 1973).

Four species are morphologically similar to *Vanderbylia cinnamomea: V. borneensis*, *V. devians*, *V. subincarnata,* and *V. ungulata*. *Vanderbylia*

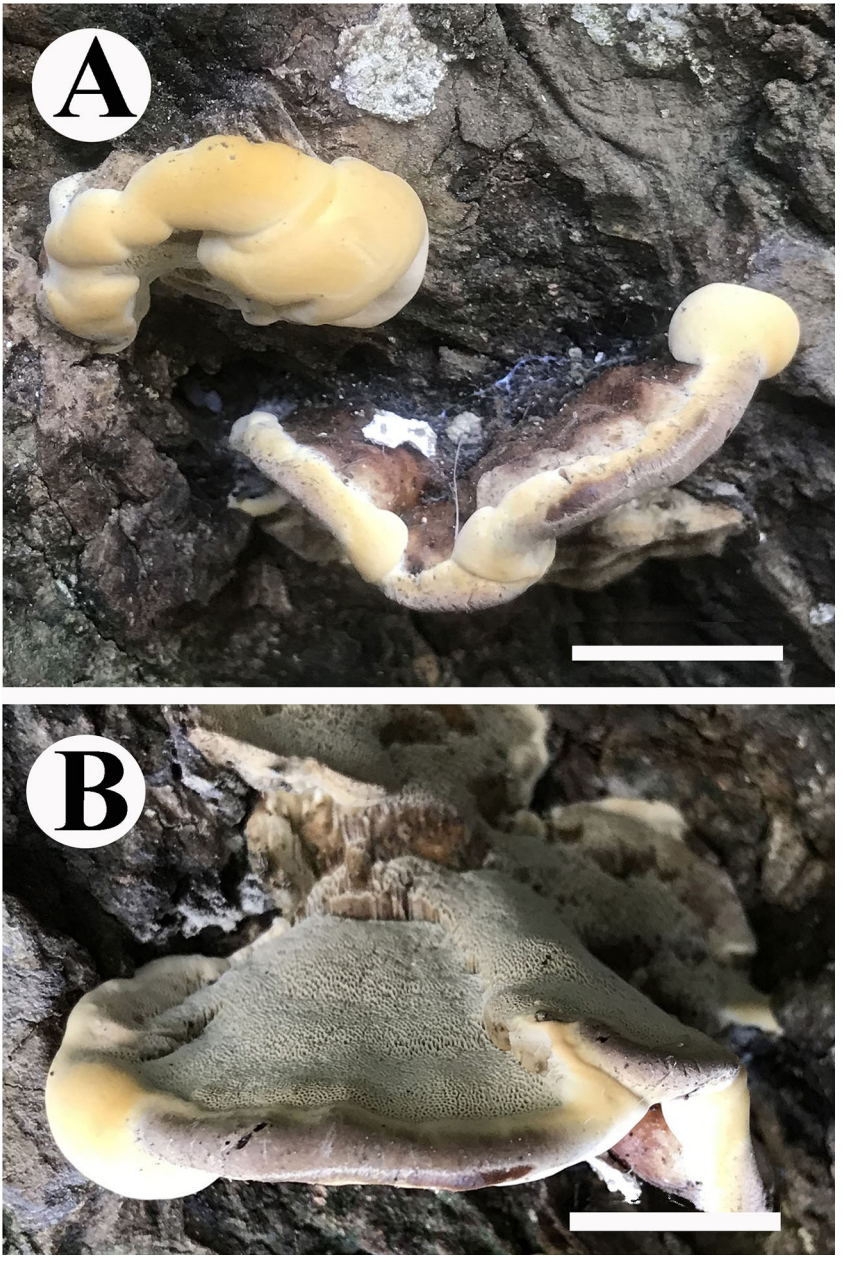


Fig. 2. *Vanderbylia cinnamomea* (holotype, SWFC 008952). Basidiocarps. Scale bars = 5 cm.

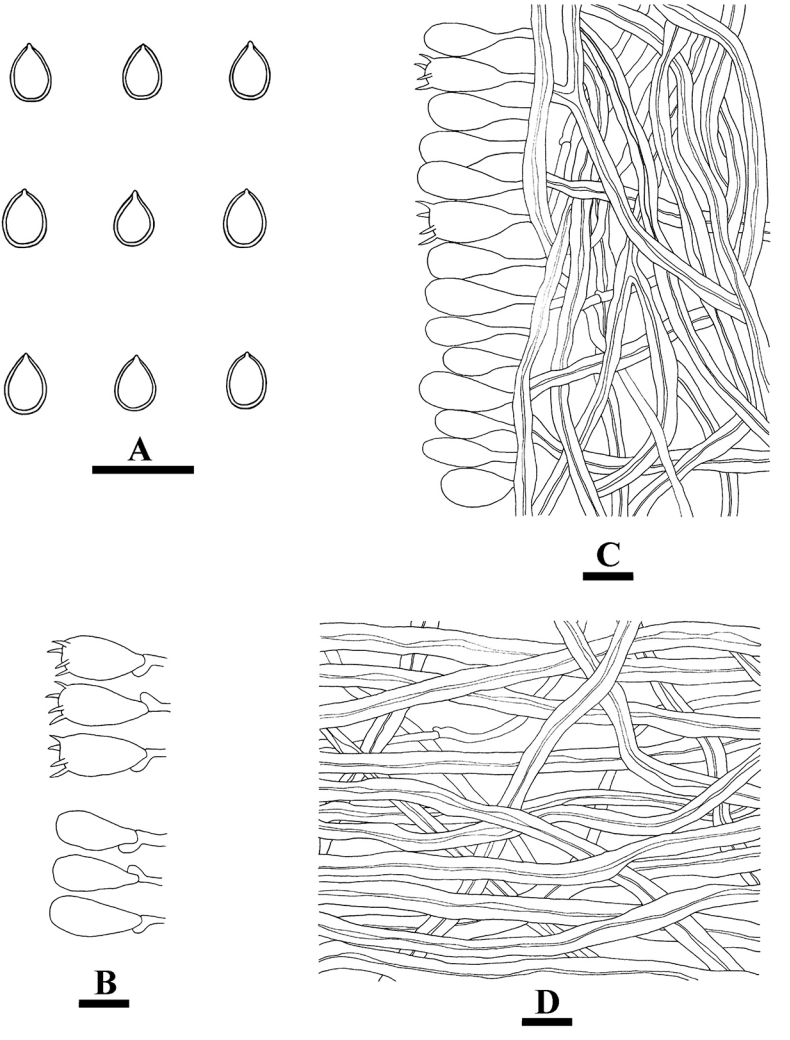


Fig. 3. *Vanderbylia cinnamomea* (holotype, SWFC 008952).
A. Basidiospores; B. Basidia and basidioles; C. Hyphae from trama; D. Hyphae from context.
Scale bars = 10 μm.

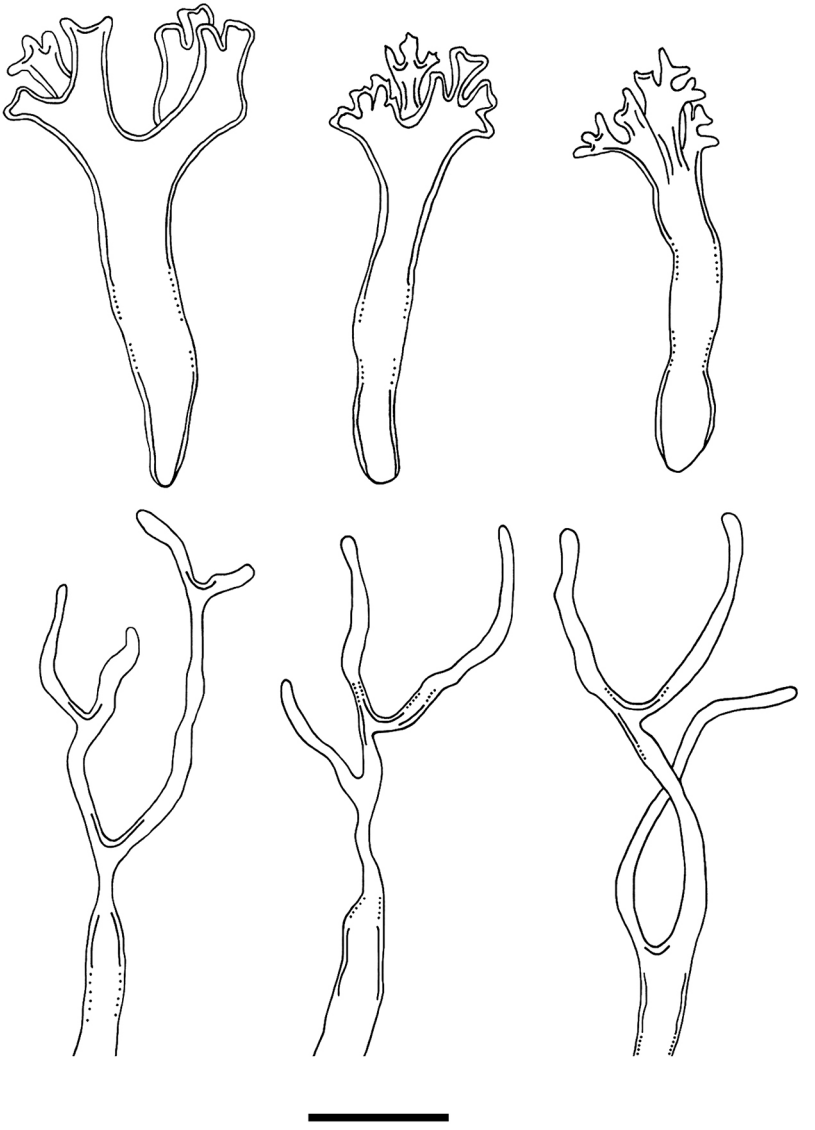

Fig. 4. *Vanderbylia cinnamomea* (holotype, SWFC 008952).
Arboriform hyphae. Scale bar = 10 μm.

*borneensis* differs from *V. cinnamomea* in its smaller pores (8–10 per mm) and the presence of skeletal hyphal endings in the dissepiments (Corner 1987); *V. devians* is distinguished by its substipitate basidiocarps with alutaceous to isabelline pore surface and larger pores (3–4 per mm, Bresadola 1920); *V. subincarnata* is diagnosed by its pinkish pore surface and smaller pores (7–10 per mm, Corner 1987); and *V. ungulata* has ungulate basidiocarps with an ochraceous pore surface and hyaline to yellow basidiospores (Ryvarden & Johansen 1980).

When Decock & Ryvarden (1999) examined the holotype of *Polyporus delavayi* Pat., they proposed that it be transferred to *Perenniporia* based on its strongly dextrinoid, cyanophilous skeletal hyphae and dextrinoid, thick-walled basidiospores; they also examined the type specimen of *Perenniporia formosana* T.T. Chang and synonymized it under *P. delavayi* (Pat.) Decock & Ryvarden. Cui & al. (2019) subsequently transferred *Polyporus delavayi* to *Vanderbylia* based on molecular data and morphological characters on finding *P. delavayi* nested within the *Vanderbylia* lineage and manifesting morphological features consistent with the concept of *Vanderbylia*. Morphologically, *V. delavayi* differs from *V. cinnamomea* by its dirty greyish orange to greyish brown pore surface, larger pores (4–5 per mm), and larger basidiospores (5.7–7 × 4.7–5.9 µm, Decock & Ryvarden 1999).

Wood-rotting basidiomycetes have been extensively studied (Gilbertson & Ryvarden 1987, Núñez & Ryvarden 2001, Bernicchia & Gorjón 2010, Dai 2012, Ryvarden & Melo 2014), but Chinese wood-rotting fungi diversity is still not well known, especially in the subtropics and tropics. The new species *Vanderbylia cinnamomea* is from the Chinese subtropics, where many new taxa in *Polyporales* have been described (Cui & al. 2007, 2011; Cui & Dai 2008; Du & Cui 2009; Li & Cui 2010; He & Li 2011; Jia & Cui 2011; Yu & al. 2013; Yang & He 2014; Chen & al. 2015; Zhao & Wu 2017; Shen & al. 2018; Zhao & Ma 2019). We anticipate that more new polypore taxa will be found in China after further investigations and molecular analyses.

**Acknowledgments**

Special thanks are due to Drs. Shah Hussain (University of Swat, Pakistan) and Yuan-Yuan Chen (Henan Agricultural University, P.R. China) who reviewed the manuscript. The research was supported by the National Natural Science Foundation of China (Project No. 31700023) and the Science Foundation of Yunnan Department of Education (2018JS326) and the Key Laboratory of State Forestry Administration for Highly Efficient Utilization of Forestry Biomass Resources in Southwest China (Southwest Forestry University) (Project No. 2019-KF10).

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